Supplemental Document

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Method for controlled tissue theranostics using a single tunable laser source: supplement

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1. 2-photon diagnostics capability of tunable MOPA laser system

It has been shown recently that FLIM can be done also by a two-photon excitation typically using Ti:sapphire femtosecond lasers, not changing fluorescence lifetime decays [1,2]. The same has been confirmed in one of our recent studies using the custom-designed MOPA GS fiber laser, but at much longer, ps pulse regimes, more qualified for the localized tissue treatment [3]. In this study, we test the diagnostics capabilities of a newly designed tunable laser system that has been found particularly applicable for the customized treatment (Fig. 3). 2-photon diagnostics was tested on the same retinal tissue used for the treatment measurements. By setting the fastest pulse repetition rate of v = 40 Mhz, MOPA laser was found capable of 2-photon diagnostics, not only AF, but also much more revealing, yet time-consuming FLIM (Fig. S 1). Laser setting with peak irradiance $j = 3*10^8$ W/cm^2, fluence ε = 0.015 J/cm^2 and dose E = 80 µJ per pixel did not cause any noticeable treatment effect. The comparison with 1-photon excitation revealed even more contrast in the detected local microenvironment but on the price of few orders of magnitude more power/energy and exposure time necessary to gain a similar signal-to-noise. If the laser parameters for diagnostics could be set to prevent tissue changes, not creating treatment effect, such a system would be considered highly prospering for future theranostic applications.

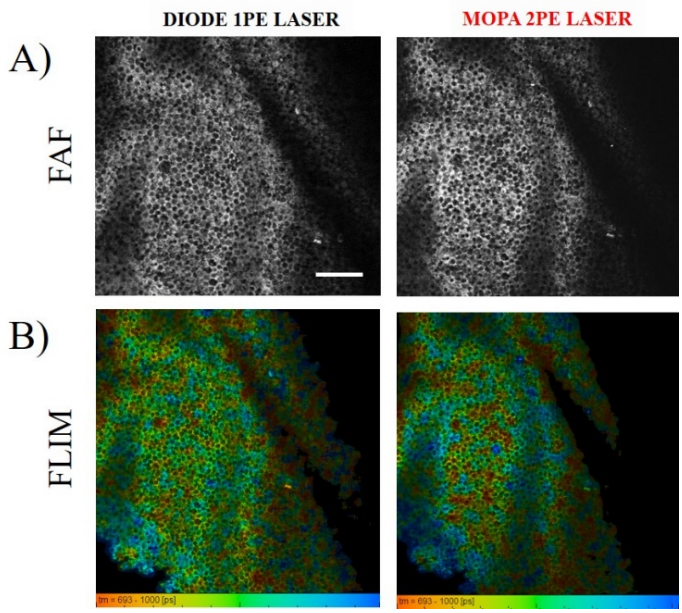

Fig. S 1. Capability of 2-photon excitation (2PE) diagnostics of tunable MOPA laser system through AF (A) and FLIM (B) imaging (right column). Comparison on the same retinal site is done with standard 1-photon excitation using pulsed diode laser (left column). Laser parameters for 1PE FLIM diagnostics were: λ= 561 nm, P = 10 µW, v = 80 MHz, pulse duration 100 ps and integration time t = 1 min. Laser parameters used for 2PE FLIM diagnostics: λ = 1030 nm, P = 170 mW, v = 40 MHz, pulse duration 70 ps, 512*512-pixel size and integration time t = 3 min. Scale bar is 100 µm.

2. Optimization of double-exponential fitting of the fluorescence lifetime of retinal tissue

Due to local deviations in the fluorescence lifetime of the retinal tissue, a special care of the fitting approach was required to be robust enough to give representative and valuable information of the system through fitted descriptors. Two approaches were tested on the retinal treatment site with changing laser dose: first by fitting all four parameters, a_1, τ_1, a_2 and τ_2, and second, by fitting only a_1 and a_2 with τ_1 and τ_2 being fixed to the mean values obtained from the τ distribution across the field of view (Fig. S 2). It was found that the fixation of lifetime values didn't particularly affect least-square fits in both, normal - untreated and altered - treated site (see the χ^2 distributions on the right side and in the Table S1 showing mean χ^2 in the different stages of treatment effect presented in Fig. 3 A). Average χ^2 remained around value 1 also when the lifetime parameters were fixed. Even more, it seems that molecular changes in retinal pigment epithelium were even more pronounced through FLIM when setting parameters fixated (more pronounced blue color in the treatment region). It means that the later fitting approach gave us even better sensitivity in diagnosing molecular changes in the tissue due to laser treatment.

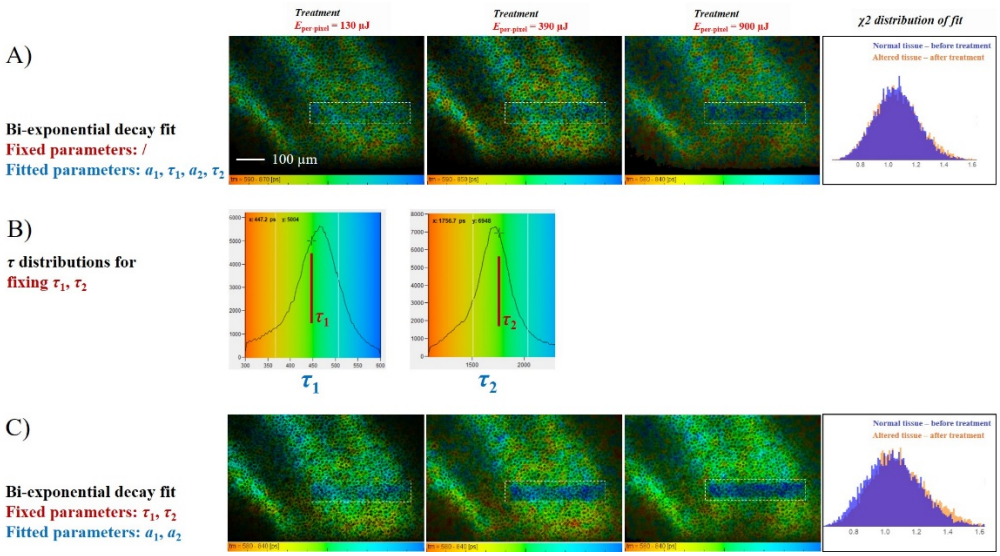

Fig. S 2. Comparison between two approaches of double-exponential fluorescence lifetime fitting. *A)* no fixed parameters *with corresponding* τ *distributions in B) and C) fixed* τ *parameters obtained from the distribution mean value.* Color coding shows mean lifetime τ_m. Fixated lifetimes values in the second approach were τ_1=450 ps and τ_2=1750 ps.

Table S 1. Mean τ_m and χ^2 with stdev in different stages of the laser treatment effect (see Fig. 3 A) in the treated and untreated region using fixed lifetime values τ_1=450 ps and τ_2=1750 ps.

Treatment effect (1-5)*	$\tau_{m\ (untreated\ site)}$	$\tau_{m\ (treated\ site)}$	$X^2_{(untreated\ site)}$	$X^2_{(treated\ site)}$
1	680 ± 40 ps	730 ± 40 ps	1.0 ± 0.15	1.0 ± 0.15
3	690 ± 40 ps	800 ± 40 ps	1.0 ± 0.15	1.05 ± 0.15
4	680 ± 40 ps	770 ± 40 ps	1.1 ± 0.15	1.1 ± 0.15
5	680 ± 40 ps	880 ± 50 ps	1.15 ± 0.15	1.15 ± 0.15

* qualitative estimation of the treatment effect (see Fig. 3 A)

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